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AN ASSESSMENT OF SEDIMENT AND BLUE MUSSELS (*Mytilus edulis*) FROM ATLANTIC CANADA HARBOURS: BUTYLTIN AND HEAVY METAL CONCENTRATIONS AND MUSSEL BIOMARKERS

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An Assessment of Sediment and Blue Mussels (*Mytilus edulis*) from Atlantic Canada Harbours: Butyltin and Heavy Metal Concentrations and Mussel Biomarkers

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ABSTRACT

One year prior to the January 1, 2003 ban on the use of tributyltin (TBT) in antifouling paints, sediment and blue mussel (*Mytilus edulis*) from harbours in St. John's, Newfoundland, and Halifax, Nova Scotia, which have large vessel traffic and repair activities, as well as two pleasure craft marinas on the south shore of Nova Scotia, were analyzed for butyltin and a suite of heavy metals. TBT concentrations in Halifax Harbour were highly variable (42-46,267 ng Sn/g dry weight), with the higher values found near ship repair yards and large vessel docks. Compared with samples collected at the same locations in 1988 and 1994, 50% of the 2002 samples had higher TBT concentrations. St. John's Harbour TBT concentrations were generally lower than those in Halifax, ranging from <1.0 to 866 ng Sn/g (mean 337 ng/g). Concentrations of TBT were generally lower at the pleasure craft marinas (43-212 ng/g) than in the large vessel harbours, but were greater than those found at the same sites in 1988 and 1994. Conversely, mussel TBT concentrations were lower in both Halifax Harbour (1.6-3.6 ng Sn/g) and St. John's Harbour (1.4-4.8 ng Sn/g) than concentrations measured in other years and locations. Concentrations of TBT were also two to three orders of magnitude lower than those found in Halifax Harbour mussels by other researchers in 1995. Based on the sediment TBT concentrations, the risk to benthic organisms, particularly those in the large vessel harbours, remains high.

Concentrations of other metals in sediments were much higher than those collected at a reference site. When results were compared with the Canadian Environmental Quality Guidelines for marine sediment, 59-94% of samples exceeded the guidelines for As, Cd, Cu, Pb and Zn, and 65-82% exceeded the probable effects level for Cu, Pb and Zn. Concentrations of most metals in sediment and mussels were similar to those found in previous years and other locations that were subject to similar inputs.

Mussels were also analyzed for various biomarkers, including vitellin-like proteins which are indicators of potential endocrine disruption, as well as changes in condition index, metallothioneins, labile zinc levels and lipid peroxidation levels, which are indicators of exposure to contaminants. Results of biomonitor analyses were correlated with some metal concentrations. Condition indices had a negative correlation with tissue copper concentration, and a positive correlation with tissue DBT and arsenic concentration, however no correlations between vitellin-like protein concentration and tissue metal concentration were found. There was no correlation between metal concentration and metallothioneins, lipid peroxidation or labile zinc concentration.

RÉSUMÉ

Depuis le 1er janvier 2003, l'utilisation du tributylétain (TBT) dans les peintures antisalissures est interdite. Un an avant la mise en œuvre de cette interdiction, on a recherché la présence de butylétain et de divers métaux lourds dans les sédiments et les moules bleues (*Mytilus edulis*) des ports de St. John's (Terre-neuve) et de Halifax (Nouvelle-Écosse), dans lesquels on observe un trafic de gros navires et des activités de réparation, et de deux marinas de plaisance situées sur la côte sud de la Nouvelle-Écosse. Les concentrations de TBT dans le port de Halifax étaient très variables (42 à 46,267 ng Sn/g de poids sec), les valeurs les plus élevées ayant été relevées près des chantiers navals et des quais pour gros navires. Par comparaison avec les échantillons prélevés aux mêmes endroits en 1988 et 1994, la moitié (50 %) des échantillons prélevés en 2002 avaient une concentration de TBT plus élevée. Les concentrations de TBT mesurées dans le port de St. John's étaient en général plus faibles que dans le port de Halifax, allant de moins de 1,0 à 866 ng Sn/g (moyenne de 337 ng/g). Les concentrations de TBT étaient en général plus faibles dans les marinas de plaisance (43 à 212 ng/g) que dans les ports pour gros navires, mais plus élevées que celles mesurées aux mêmes sites en 1988 et 1994. Réciproquement, les concentrations de TBT dans les moules étaient moins élevées dans le port de Halifax (1,6 à 3,6 ng Sn/g) et le port de St. John's (1,4 à 4,8 ng Sn/g) que celles mesurées au cours d'autres années et ailleurs. Les concentrations de TBT étaient également de deux à trois ordres de grandeur plus faibles que celles mesurées dans les moules du port de Halifax par d'autres chercheurs en 1995. D'après les concentrations de TBT dans les sédiments, le risque pour les organismes benthiques, en particulier ceux se trouvant dans les ports pour gros navires, demeure élevé.

Les concentrations d'autres métaux dans les sédiments étaient beaucoup plus élevées que celles mesurées dans les sédiments prélevés dans un site de référence. Lorsque les résultats sont comparés aux *Recommandations canadiennes pour la qualité de l'environnement* visant les sédiments marins, on relève des concentrations supérieures aux recommandations pour les métaux (As, Cd, Cu, Pb et Zn) dans 59 à 94 % des échantillons, et dans 65 à 82 % des échantillons, la concentration est supérieure à la concentration avec effet probable pour le Cu, le Pb et le Zn. Les concentrations de presque tous les métaux dans les sédiments et les moules étaient similaires à celles relevées les années précédentes à d'autres lieux exposés aux mêmes apports.

Les chercheurs ont aussi mesuré d'autres biomarqueurs chez les moules, notamment les protéines de type vitelline qui sont des indicateurs de perturbation endocrinienne potentielle, les modifications de l'indice de condition, les métallothéionéines, les taux de zinc labile et les taux de peroxydation lipidique, qui sont des indicateurs de l'exposition aux contaminants. Les résultats des analyses de biosurveillance ont été corrélés aux concentrations de métaux. On a relevé une corrélation négative entre l'indice de condition et la concentration de cuivre dans les tissus, et une corrélation positive entre la concentration de DBT et d'arsenic dans les tissus, mais aucune corrélation n'a été observée entre la concentration de protéines de type vitelline et la concentration de métaux dans les tissus. Aucune corrélation n'a été relevée entre la concentration de métaux et les métallothéionéines, la peroxydation lipidique ou la concentration de zinc labile.

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1.0 INTRODUCTION

Tributyltin (TBT) has been described as "perhaps the most toxic chemical that has ever been deliberately introduced into the aquatic environment" (Maguire 2000). While best known for its use in antifouling paints on ships, TBT is also used as a slimicide in cooling towers, as a preservative for wood, textiles, paper and leather, as stabilizing material in polyvinyl chloride pipes, and as a catalyst for synthesis of polyurethane foam and silicone rubber (summarized by Dubey & Roy 2003). In Canada, its main uses are as a PVC stabilizer (67% of market), industrial catalyst (8% of market) and biocide, wood preservative and slimicide (20% of market) (Environment Canada 1993).

Widespread commercial production of TBT started in the late 1960s, when it was determined to be highly effective in the prevention of biofouling on ship hulls, marine structures and fishing nets. Prior to the 1960s, antifouling paints were largely based on copper(I) oxide and non-tin organometallic compounds (Evans 1999).

The harmful effects of TBT were first detected in Arcachon Bay, Western France, where it was linked to oyster shell malformation, poor spatfall, and the near-collapse of the local oyster-farming industry (Alzieu 1980). Shortly thereafter, imposex (superimposed male genitalia in females) due to TBT exposure was discovered in dogwhelk populations in south-west England (Bryan et al. 1986). These events triggered surveys of TBT pollution worldwide that indicated that the problem was global. It became clear that TBT can leach from paints into the water column and adsorb strongly to sediments. While its half-lives in water are days or weeks, its half life in sediment may be a matter of years (Maguire 2000). Both acute and chronic toxic effects including reproductive failure, growth inhibition and death were found in a large number of marine organisms, ranging from benthic invertebrates to molluscs and fish. Imposex has been detected in over 140 species of gastropods alone (Omae 2003). More recently, the known toxicity of TBT has expanded to include immunotoxic and genotoxic effects on fish and other species (e.g. Grundler et al. 2001, Patricolo et al. 2001, Tiano et al. 2001). Furthermore, many studies have demonstrated the tendency of aquatic organisms to bioconcentrate TBT from water and sediment, with bioconcentration factors often in the thousands and tens of thousands (Maguire 2000). The biocide's potential to biomagnify in higher trophic levels is indicated by the presence of TBT and its degradation products in the tissues of top predators including sea birds, dolphins, whales and even humans (reviewed by Maguire 2000).

In 1982, France was the first country to regulate the use of organotin-based antifouling paints, prohibiting their use on vessels less than 25 m in length (Alzieu 1991). Many countries later followed suit, including Canada (in 1989), the United States, Australia, New Zealand, South Africa, Hong Kong, and most European countries. Legislation also required the use of self-polishing copolymer (SPC) paints, which leach TBT at a slower rate than "free association" paints (Evans et al. 2000).

An abundance of research has been conducted since the implementation of TBT-controlling legislation to monitor its efficacy. Results have been mixed, with many studies demonstrating substantial reductions in concentrations of TBT in the water column, sediments and tissues of biota worldwide, and recovery of many populations of organisms affected by TBT (reviewed by Evans 1999). However, an apparently equal number of studies state the opposite: that TBT residue concentrations and/or associated toxic effects have not declined markedly (e.g. Minchin et al. 1995, Smith 1996, Michel et al. 2001).

In Canada, a survey of water and sediment from across Canada showed that the 1989 regulation had only been partly effective. It had little effect in the reduction of TBT concentrations in seawater and sediments, and in many locations, concentrations were high enough to cause acute and chronic toxicity to aquatic and benthic organisms (Chau et al. 1997a). Furthermore, whelk populations in some British Columbia harbours still showed a near 100% incidence of imposex in 1994 (Tester et al. 1996), and residues of TBT in four species of mussel from across Canada were similar to those measured in other parts of the world before regulations were implemented (Chau et al. 1997b).

TBT pollution and effects in Atlantic Canada have been comparable to those found across Canada and worldwide. TBT monitoring during the past 15 years has included the analysis of sediment concentrations (Julien et al.1991, Hennigar & Garron 1992, Chau et al. 1997a, Ernst et al. 1999), water concentrations (Julien et al. 1991), sandblasting grit concentrations (Hennigar & Garron 1992), mussel tissue concentrations (Chau et al. 1997b), mussel toxicity (Julien et al. 1991) and dogwhelk imposex (Prouse & Ellis 1997). The overall conclusion from these studies was that TBT still presents a significant environmental problem, particularly in large-vessel harbours.

By the late 1990s, it appeared that fresh TBT inputs were being received by harbours that handle large commercial vessels legally painted with TBT. This prompted the International Maritime Organization (IMO) to adopt a resolution in 1998 to phase out all use of organotin antifouling paints by January 1, 2003 (Maguire 2000). This included a review of the persistence, bioaccumulation and toxicity of TBT in aquatic environments, which concluded that TBT meets all criteria for designation as a Track 1 substance under Canada's Toxic Substances Management Policy, necessitating measures to virtually eliminate it from the Canadian environment. As a result, the Pest Management Regulatory Agency (Health Canada) stopped sales of TBT-based antifouling paints on December 31, 2002.

This report summarizes the results of a monitoring program carried out by Environment Canada in 2001/2002 to assess the levels of TBT contamination and corresponding environmental risks in the year prior to the implementation of additional controls. The study examined sites in Halifax harbour and two pleasure craft marinas as well as St. John's harbour, an area where few TBT studies have been conducted in the past. In addition, heavy metal concentrations were analyzed in environmental samples. Various physiological and morphometric characteristics of mussels were also determined. Those included: vitellin-like proteins, metallothioneins, labile zinc and lipid peroxidation levels as well as condition index.

2. 0 METHODS

2.1 Sediment Sampling

In December 2001 and January 2002, sediment samples were collected from the locations listed in Tables 1 and 2 (see also Figs. 1 to 5). Additional samples were collected in March 2003 from the Halifax Shipyard site and Chezzetcook Inlet.

Table 1. Sediment sample locations in St. John's Harbour, Newfoundland.

Location	Activity [approximate # of vessels]	Max Vessel Size
Small boat basin West	Docking for pleasure & small fishing boats [>50 pleasure craft].	<25m (40-50')
Small boat basin East	Docking for pleasure & small fishing boats [>50 pleasure craft].	<25 m (40-50')
Graving dock	Ship repair, sand blasting, painting [4 ships].	>25m (150')
Synchro Lift	Ship repair, sand blasting, painting.	>25 m (150')
Murray Premises	Docking for larger ships.	>25 m (500')
Pier 18	Docking for larger ships.	>25 m (1000')
Middle of harbour	Shipping lane for small and large vessels.	<25 m & >25 m
Oceanex Pier	Docking for large maritime transporters [3-5 ships].	>25 m (500')

Table 2. Sediment sample locations in Nova Scotia.

Location	Activity [approximate # of vessels]	Max Vessel Size
Dartmouth Marine Slips	Ship repair (sand blasting, painting) for over 50 years until late 1990s. Some small boat moorings. Sewage outfall nearby.	<25 m (50-60') >25 m (150')
Shearwater	Moorage for domestic & foreign naval vessels and pleasure craft. Sewage outfall within ½ km [>100 pleasure craft and 3 ships].	<25 m (50-60') >25 m (100')
Synchro Lift	Ship repair (sand blasting, painting) for over 25 years until late 1990s.	>25 m (150')
Halifax Shipyard	Ship building, repair and moorage for over 100 years [3-4 ships]. Sewage outfall nearby.	>25 m (400-500')
Bedford Basin	Some small boat traffic, no moorage nearby.	<25 m
South Shore Marina	Pleasure craft moorage for over 30 years [150 pleasure craft].	<25 m
Oak Island Marina	Pleasure craft moorage for over 30 years [70 pleasure craft].	<25 m
Chezzetcook Inlet (reference site)	Area of negligible amounts of boating and moorage.	<8 m (25')

Figure 1: Study Locations in Atlantic Canada

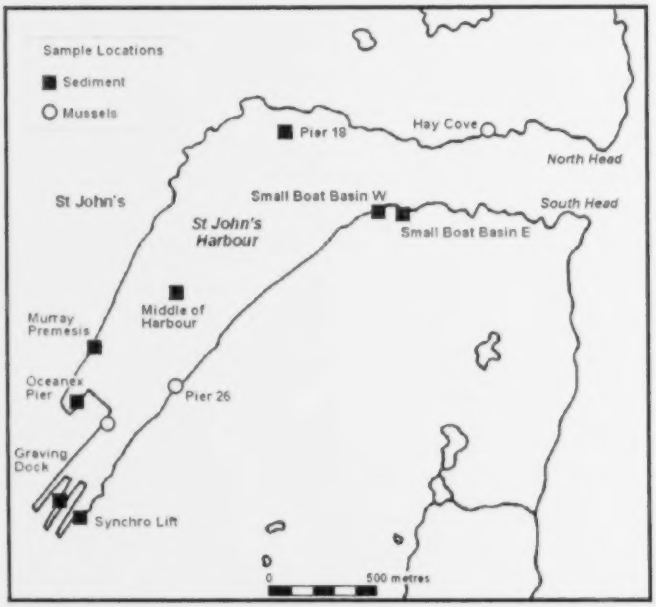

Figure 2: Sediment and Mussel Sample Locations in St. John's Harbour

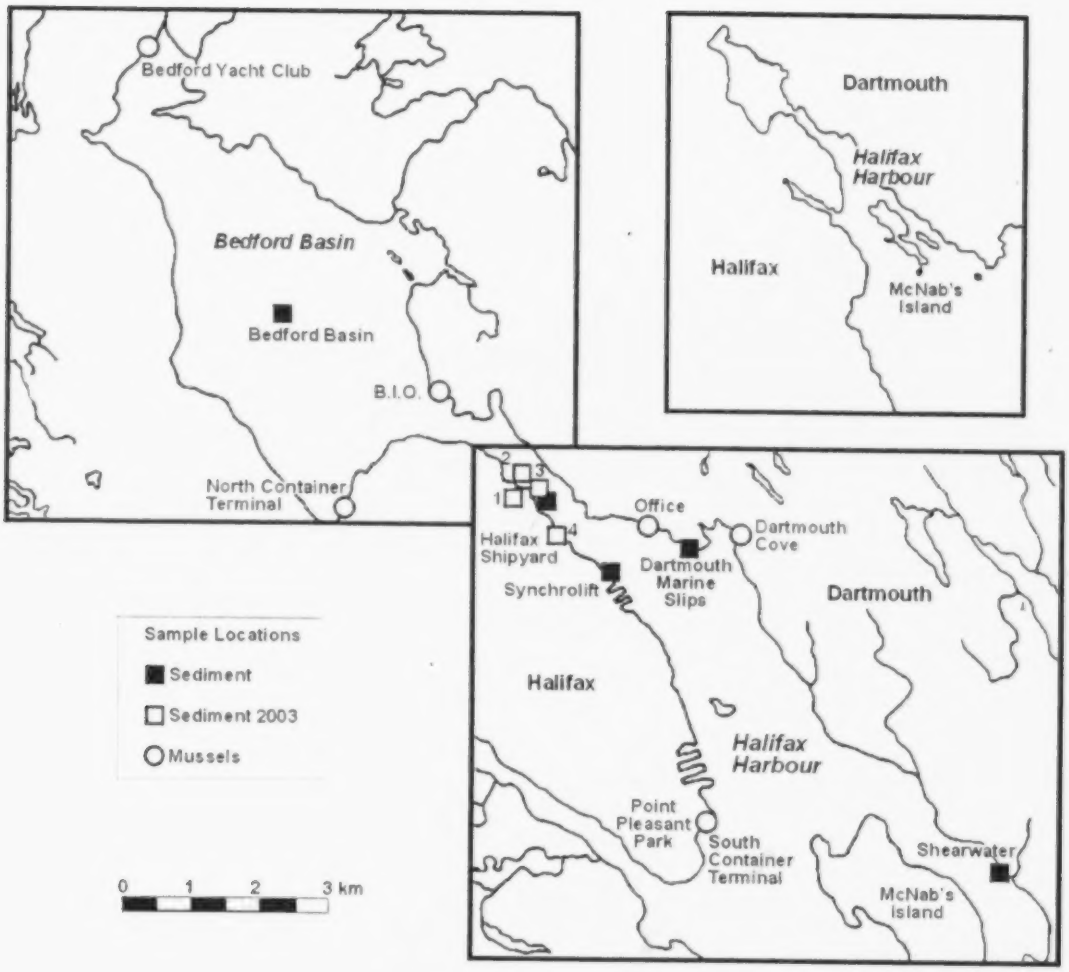

Figure 3: Sediment and Mussel Sample Locations in Halifax Harbour

Figure 4: Sediment Sample Locations, South Shore, Nova Scotia

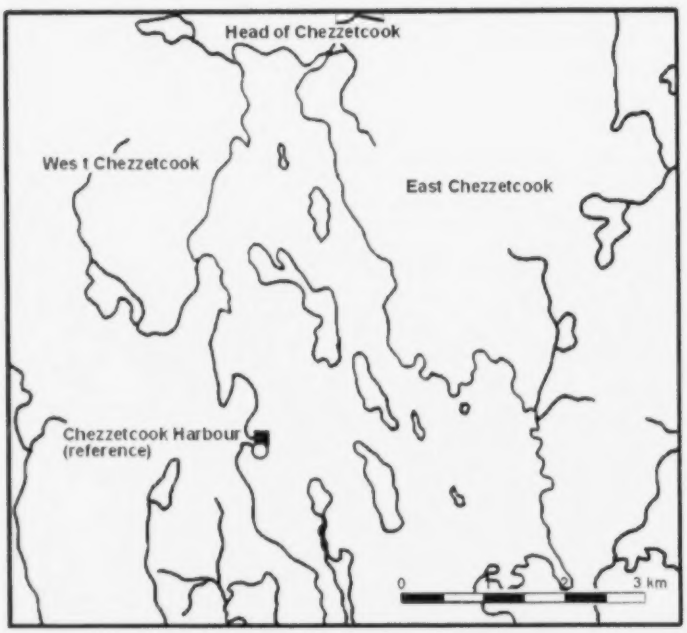

Figure 5: Reference Sediment and Mussel Sample Locations, Nova Scotia

Sediment samples were collected using a Phleger gravity core device with a Teflon liner (11½ x 1½") and stainless steel cutting head/retainer. At each sample site, duplicate cores of at least 6-7 cm were obtained. The cores were maintained upright and frozen within one hour of collection. The samples were kept frozen for several days, before being removed from the Teflon tubes. The top 3 cm were removed with a stainless steel blade. The samples were allowed to thaw and duplicate samples were combined, divided into two equal aliquots and placed in new glass bottles with Teflon caps. Within one week, samples were sent to the Research Productivity Council (RPC) laboratory, Fredericton, NB, for butyltin analysis and the Environment Canada laboratory, Moncton, NB, for metals analysis.

2.2 Mussel Sampling

In December 2001 and January 2002, mussel samples were collected from the locations listed in Table 3 (see also Figs. 1-3, 5).

Table 3. Mussel sample locations in St. John's Harbour, Newfoundland (SJH); Halifax Harbour, Nova Scotia (HH); and Chezzetcook Inlet, Nova Scotia.

Location	Description	Activity (approx. # of vessels)	Max Boat Size
SJH – Pier 26	S side of harbour, at CFS St. John's	Docking for larger ships.	>25 m (up to 1000')
SJH – Hay Cove	N side of harbour, outside narrows	No fishing or anchorage.	n/a
SJH – Pier 3	S end of harbour, near New Dock ship repair & Oceanex container port	Docking for larger ships.	>25 m (up to 1000')
HH – Bedford Yacht Club	S side of property near rock/concrete wall	Pleasure craft moorage (~150) within 100' of site. Near Sackville River drainage.	<25 m
HH – Dartmouth Cove	S of Coast Guard Base and Dartmouth Marine Slips	Near sites where large vessels moored, sandblasted and painted. Nearby sewage outfall.	>25 m (up to 500')
HH – Office	S of Macdonald Bridge, E side of harbour	Pleasure craft moorage nearby and sewage outfall within 100 m.	<25 m
HH – B.I.O.	E side of harbour, within BIO small boat jetty	Moorage for several small boats, area of limited water circulation.	<25 m (40-60')
HH – N Container Terminal	W side of harbour, in Bedford Basin	Site of large vessel traffic and moorage for over 25 years.	>25 m (up to 1000')
HH – S Container Terminal	W side of harbour, south end Halifax	Site of large vessel traffic and moorage for over 40 years.	>25 m (up to 1000')
Chezzetcook Inlet (reference site)	30 km east of Halifax	Area of negligible amounts of boating and moorage.	n/a

Samples were collected within one hour of low tide from locations near the water's edge. At each location, approximately 100 mussels ranging from 3 to 5cm in length were collected by hand and placed in polyethylene bags. They were frozen within one hour of collection and then

sorted for size and distribution to the appropriate laboratory: RPC in Fredericton, NB for butyltin analysis, the Environment Canada laboratory in Moncton, NB for metals analysis, and the Environment Canada St. Lawrence Centre laboratory in Montreal, QC for biomarker analysis.

2.3 Sediment and Mussel Butyltin Analysis

Analysis of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) in sediment and mussels was conducted according to Hennigar (1990). Sediment samples were air-dried and ground with anhydrous sodium sulphate. Dried sediment was refluxed with a tropolone/benzene solution, then cooled, filtered, and reduced in volume under nitrogen at 40-50°C to 1 mL. Mussel homogenates were digested with hydrochloric acid (HCl) and the hydrophobic component separated from a mixture of water, sodium chloride and tropolene or benzene solution. Organotins in the sediment/biota concentrates were ethylated with 1 M ethyl magnesium bromide in tetrahydrofuran solution. After neutralization with HCl solution, and separation using hexane, the extract was cleaned on a glass column containing 5% deactivated silica gel and a layer of sodium sulphate. The column was eluted with hexane and the eluent reduced to approximately 900 µl on a nitrogen evaporator, centrifuged, and transferred to a GC vial. Butyltins were analyzed using a Hewlett Packard 6890 gas chromatograph with autosampler and an HP G2350A atomic emission detector. Results were reported as dry weight for sediment samples, and wet weight for mussel samples. All blank and solvent spike results were within acceptable limits, with the exception of one monobutyltin spiked sample, which was associated with an isolated matrix effect on the retention time.

2.4 Sediment & Mussel Metal Analysis

Sediment samples were prepared for analysis using a microwave-assisted acid digestion (MARSx) method (EPA 3051). Approximately 0.15 g of wet sediment along with 10 mL HNO^3, 1 mL H^2O^2, 1 mL HCl and 5mL deionized water were placed in a reference vessel on an XP-1500 *Plus* support module and the samples irradiated to 170-180°C in a microwave (CEM MARSx) for approximately 35 minutes. After venting of the vessels, samples were placed in 50 mL graduated vials with screw caps, diluted to 50 mL and shaken to homogenize. The samples were then centrifuged at 1500 rpm for 5 minutes and diluted 1:10 in 15 mL vials. Samples were analyzed for silver, arsenic, cadmium, cobalt, chromium, iron, manganese, molybdenum, nickel, lead, antimony, tin, vanadium and zinc concentrations using ICPMS (Elan 6000). Results were reported as dry weight following determination of moisture content in the samples. Mussel samples were prepared for analysis using a microwave-assisted acid digestion (MARSx) method (EPA 3052). This method is similar to that for sediments with the following exceptions: mussel samples were freeze-dried prior to digestion; tissues were digested in HNO_3 and H_2O; and the microwave program was shorter. Samples were analyzed for arsenic, cadmium, cobalt, copper, manganese, nickel, lead, selenium and zinc concentration using ICPMS (Elan 6000). Results for both sediment and mussels were reported as dry weight. Method and instrument blank and reference sample results were within acceptable limits.

2.5 Biomarker Analysis

Prior to analysis, total weight, length and shell weight were recorded for each mussel. Sex determination of most mussels was not possible because the gonads were in an undifferentiated stage. For each biomarker, the digestive gland and/or gonad tissues of between 12 and 25 mussels (depending on sample location) were analyzed. For metallothionein (MT) and lipid peroxidation (LPO) analysis, the digestive gland (hepato-pancreas) was removed from each mussel and homogenized with a Teflon pestle tissue grinder

in ice-cold 25 mM Tris-acetate buffer (pH 7.6) containing 250 nM sucrose and 1 mM dithiothreitol at a 1:10 weight:volume. Aliquots of each homogenate were taken for total protein determinations and for measuring LPO concentrations. LPO was measured by the thiobarbituric acid method which measures the production of malonaldehyde (Wills, 1987). Concentrations of LPO were normalized with the protein concentrations of each homogenate. Remaining homogenate was centrifuged at 10,000 g for 30 min at 2°C. The supernatant was separated from the pellet and measured for MT concentrations according to spectrophotometric methodology (Viarengo et al. 1997). MT concentrations were normalized with protein concentrations in the supernatant.

Vitellin-like proteins in mussel gonads were determined using the alkali-labile phosphate principle on fractionated high molecular weight proteins reported by Blaise et al.,1999. A modified, more sensitive version was used in which lipophilic proteins were precipitated with 35% acetone (instead of ether). As sex determination in mussels was not possible because the gonads were mainly in the undifferentiated stage, the removal of background level alkali-labile phosphates in males was not possible. Labile zinc concentrations in the digestive gland and gonad tissues of mussels were assayed according to Gagné and Blaise, 1996.

3.0 RESULTS & DISCUSSION

3.1 Butyltins in Sediment

Halifax Harbour had the greatest concentrations of butyltins, followed by St. John's Harbour and the pleasure craft marinas (Table 4, Figure 6). TBT concentrations in Halifax Harbour ranged from 42 to 46,000 ng Sn/g. The highest was measured near the Halifax Shipyard, a facility where large ocean-going vessels have been built, repaired and moored for over 100 years, and the highest production facility in the Atlantic Provinces. A concentration of 1,985 ng Sn/g was found at Dartmouth Marine Slips, a medium-sized ship repair facility that has not been active since 2001, and 1,459 ng Sn/g at the National Defence Synchrolift repair facility, which has not been active in ship maintenance in recent years. Concentrations were much lower at Shearwater (42 ng/g), a berthing facility only, and Bedford Basin (57 ng/g), a control site within the industrial complex.

As the Halifax Shipyard sample had such a high concentration of butyltins, further sampling was conducted a year later at four locations on the property. TBT results (Table 4) ranged from 1,440 to 13,400 ng/g, with the highest concentration found close to the site sampled the previous year. While 2003 results were much lower than the previous result of 46,267 ng/g, they were still greater than at other locations in Halifax Harbour.

In St. John's Harbour, TBT concentrations ranged from below the limit of detection to 866 ng Sn/g. The highest concentrations were found at the Graving Dock (866 ng/g) and Synchrolift (517 and 436 ng/g) sites, both ship repair facilities. The small boat moorage site (Small Boat Basin) had a much lower sediment TBT concentration of 26 ng/g. Intermediate concentrations were found at Pier 18 (385 ng/g), a large vessel docking area, and in the middle of the harbour (126 ng/g). TBT was not detected in the sample from Murray Premises, another large vessel docking area, although DBT and MBT were present.

Table 4. Butyltin concentrations in sediments from harbours in St. John's and Halifax, as well as two Nova Scotian marinas and Chezzetcook Inlet, Nova Scotia. Includes additional samples collected at Halifax Shipyard and Chezzetcook Inlet in 2003.

Location	Butyltin (ng Sn/g dry weight)			Total Butyltin	TBT % of total
	TBT	DBT	MBT		
Halifax Harbour (2002)					
Bedford Basin (BB)	56.9	69.3	<1.0	126	45.1
Dart. Marine Slips (DM)	1985	2315	1687	5987	33.2
Shearwater (SW)	42.1	60.3	<1.0	102	41.1
Synchro Lift (SL)	1459	1022	815	3296	44.3
Halifax Shipyard (HS)	46267	5654	517	52438	88.2
Halifax Harbour Mean	9,962	1,824	604	12,390	50.4
St. Dev	20,313	2,331	699	22,522	21.7
Halifax Harbour (2003)					
Halifax. Shipyard 2003 #1	2420	92	40	2552	94.8
Halifax Shipyard 2003 #2	5290	141	24	5455	97.0
Halifax Shipyard 2003 #3	13400	326	29	13755	97.4
Halifax Shipyard 2003 #4	1440	65	23	1528	94.2
Halifax Harbour 2003 Mean	5,638	156	29	5,544	95.9
St. Dev	5,427	117	7.8	5,823	1.6
St. John's Harbour					
Small Boat Basin (SB)	26.2	429	10.4	466	5.6
Graving Dock (GD)	866	713	67.9	1647	52.6
Synchro Lift (SL)	517	379	47.9	944	54.8
Murray Premises (MP)	<1.0	8.8	45.3	54.1	0
Pier 18 (18)	385	46.8	33.3	465	82.8
S. Synchro Lift (SS)	436	379	47.9	863	50.5
Middle of SJH (MH)	126	129	79.4	334	37.7
St. John's Harbour Mean	337	298	47.4	682	40.6
St. Dev	311	251	22.4	523	29.2
Pleasure craft marinas					
South Shore Marina	212	443	75.7	731	29.0
Oak Island Marina	43.3	74.6	21.1	139	31.2
Control					
Chezzetcook Inlet	3.2	1.5	1.5	6.2	51.6
Chez. Inlet 2003 #1	1.0	<1.0	<1.0	2.0	50.0
Chez. Inlet 2003 #2	<1.0	<1.0	<1.0	<1.0	na

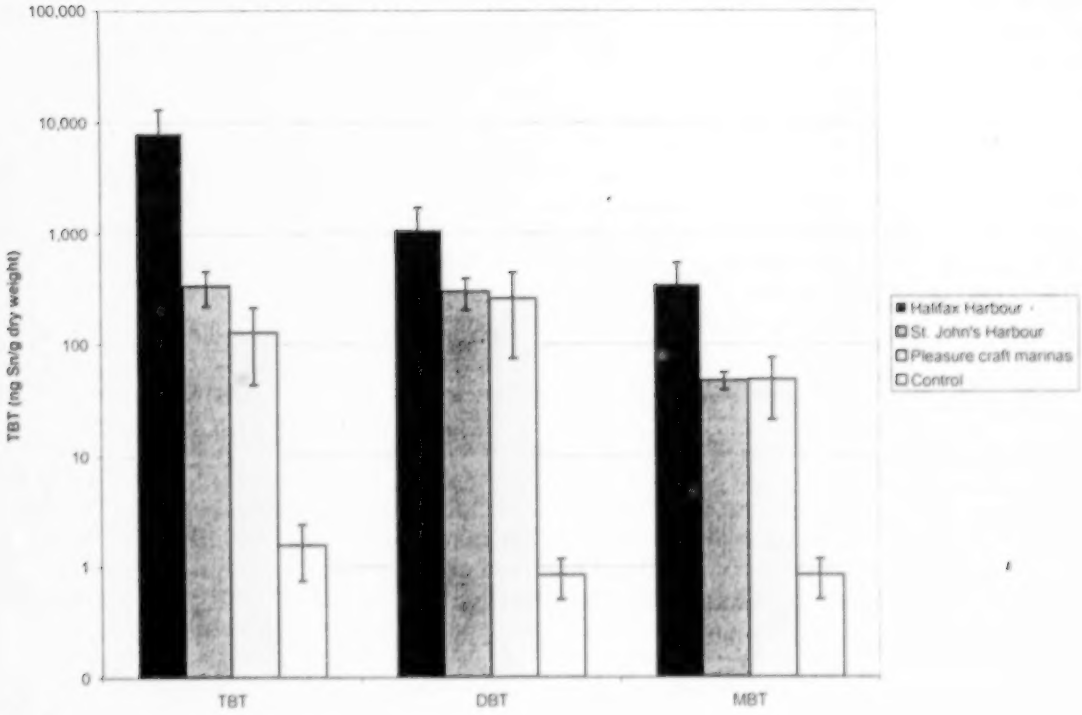

Figure 6. TBT concentrations in sediment.

TBT concentrations at South Shore and Oak Island Marinas were 212 and 43 ng Sn/g respectively, while Chezzetcook Harbour, an area of negligible boating and moorage activity, had a sediment concentration of only 3.2 ng Sn/g. Further sampling at the same Chezzetcook location in 2003 indicated a TBT concentration which was at or below the detection limit of <1.0 ng/g.

These results indicate that, in general, the degree of butyltin contamination in Atlantic Canada harbours has not declined significantly in the past decade. In Halifax Harbour, sediment samples were also collected in 1988 and 1994 in the vicinity of the current study locations (Ernst et al. 1999). While concentrations decreased between 1988 and 2002 at Dartmouth Marine Slips (Figure 7), the other three locations had higher TBT concentrations in 2002 than in either 1988 (Shearwater) or 1994 (Synchrolift) or both years (Halifax Shipyard). The most significant increase was observed at Halifax Shipyard, increasing from 66 ng/g in 1988 to 3200 ng/g in 1994 and 46,267 ng/g in 2002. These increased analytical values may be actual paint dust from the hull blasting operation that escaped the tarped enclosure and settled near the floating dock.

The mean TBT concentration in St. John's Harbour (337 ng/g from this study) was higher than in sediments collected at other St. John's Harbour locations in 1993 and 1994 (Chau et al. 1997a). Four out of seven of the 2002 samples had higher concentrations than the earlier results of 113 ng/g (1994) and 147 ng/g (1993).

The TBT concentrations also increased at the two marina sites. South Shore Marina and Oak Island Marina in 2002 compared with the same location sampled in 1988 and 1994 by Ernst et al. 1999 (Figure 8.). This is surprising considering the use of TBT-containing paints on smaller boats has been restricted for several years. However, the results may reflect an increase in boat traffic in the area.

The concentrations of TBT in Halifax and St. John's Harbour sediments, with the exception of the Halifax Shipyard samples, are similar to those in other harbours in Canada and other countries where traffic and repair of large vessels predominates (Table 5). As well, concentrations at the two marinas are comparable to TBT levels found at marinas and other small vessel areas in Canada and other countries (Table 6). The TBT concentration in sediments from small vessel marinas was substantially lower than that found in large vessel harbours.

3.1.1 Sources of TBT Release

TBT may enter the marine environment in a variety of ways. However, it is widely recognized that, even with the advent of slower-release self-polishing copolymer (SPC) paints, the main source is the leaching of TBT from paints on moored boats and boats travelling through the water. Leach rate is affected by parameters such as pH, salinity, water temperature and vessel speed (Thomas et al. 1999). In situ measurement of TBT-based paint leachates have shown that TBT (and not its degradation products) is the principal compound released in water (Clark et al. 1988).

Another tributyl tin source in the marine environment, more specifically, at industrial sites is the release of blasting residuals at ship repair facilities that are utilizing inadequate containment. A study of Atlantic Canada shipyards found TBT concentrations of over 2,000 µg/g in sandblasting grit, used by shipyards to remove TBT coatings (Hennigar & Garron 1992). While the study concluded that containment methods were useful in reducing TBT loadings to the environment, they may not be totally effective. When very high concentrations of TBT are found (e.g. >10,000 ng/g), it is thought to be due to the presence of paint chips (Sarradin et al. 1995). This may have been the case for the very high concentration found at Halifax Shipyard in this study. The TBT concentration of over 46,000 ng/g was higher than any found in the literature for other regions.

Uses of TBT in lumber preservatives, cooling tower slimicides and PVC stabilizers have resulted in the detection of organotins in municipal sewage and landfill leachates. In Switzerland, for example, butyltin concentrations in the hundreds of ng/L were found in treatment plant influents (Fent & Muller 1991). A study of sewage treatment influent, effluent and sludges in five central Canadian cities found MBT in all influent samples, but DBT and TBT only infrequently (Chau et al. 1992). The use of coated PVC pipe has led to the detection of organotin leachates in drinking water, measuring 100 ng Sn/L DBT and 13 ng/L MBT in one study (Forsyth 1997). Although such discharges may be additive to harbour sediments, they probably constitute much less mass then do the vessel antifouling uses.

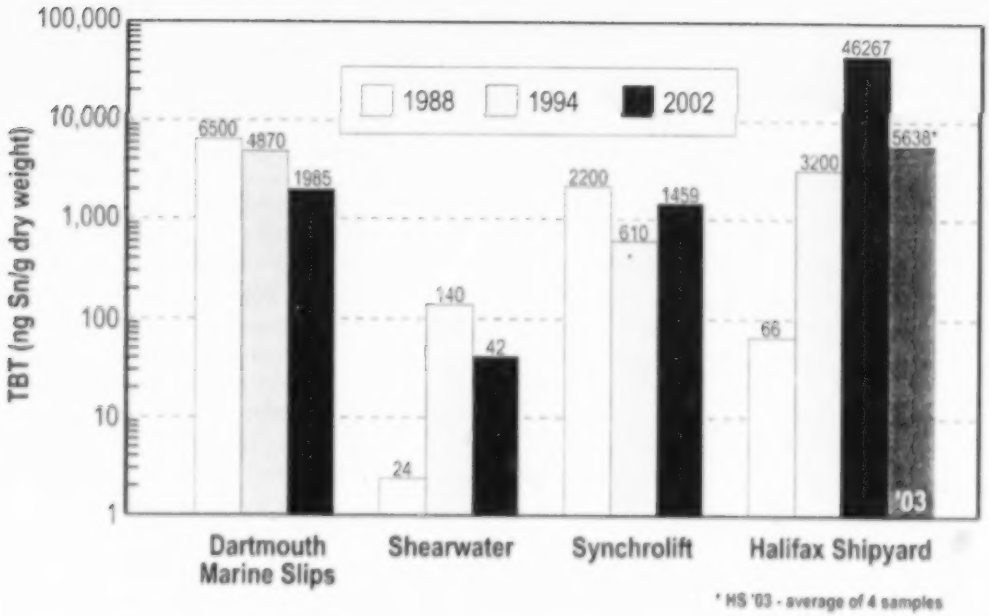

Figure 7. TBT concentrations in samples from proximal locations at Halifax Harbour sites in 1988, 1994, 2002 and 2003.

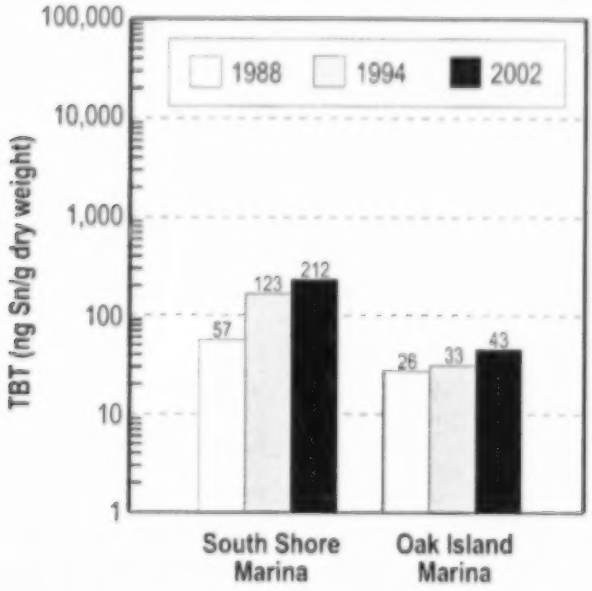

Figure 8. TBT concentrations in samples from proximal locations at South Shore and Oak Island Marinas in 1988, 1994 and 2002.

Table 5. Sediment TBT concentrations measured at large vessel harbours in this study and in others.

Sediment TBT (ng Sn/g)		# Sample Locations	Location	Year	Reference
Min	Max				
42	46267	5	Halifax Harbour, NS	2002	This work
nd	866	7	St. John's Harbour, NF	2002	This work
nd	1262	33	Prince Rupert Harbour, BC	1995	Thompson et al. 1998
140	4870	4	Halifax Harbour, NS	1994	Ernst et al. 1999
40	1909	3	Halifax Harbour, NS	1994	Chau et al. 1997
100	125	2	St. John's Harbour, NF	1994	Chau et al. 1997
	21	1	Saint John Harbour, NB	1994	Chau et al. 1997
11	2525	16	Burrard Inlet, BC	1994	Chau et al. 1997
	1348	1	Halifax Harbour, NS	1993	Chau et al. 1997
	147	1	St. John's Harbour, NF	1993	Chau et al. 1997
	3212	1	Saint John Harbour, NB	1993	Chau et al. 1997
24	6500	4	Halifax Harbour, NS	1988	Ernst et al. 1988
60	200	4	4 Harbours, Corsica	1999	Michel et al. 2001
36	4500	4	4 Harbours, Thailand	1995	Kan-Atireklap et al. 1997
665	9645	3	2 Shipyards, Maine, USA	1992	Page et al. 1996a

Table 6. Sediment TBT concentrations measured at marinas or other small vessel areas in this study and in others.

Sediment TBT (ng Sn/g)		# Sample Locations	Location	Year	Reference
Min	Max				
43	212	2	2 Marinas, NS	2002	This work
23	189	8	8 Marinas, Corsica	1999	Michel et al. 2001
9	300	11	Small boat piers, Thailand	1995	Kan-Atireklap et al. 1997
9	200	10	5 small vessel areas, NS	1994	Ernst et al. 1999
6	50	7	False Creek, BC	1994	Chau et al. 1997
21	340	5	Marinas, San Diego Bay, US	1991	Valkirs et al. 1991
6	88	9	4 small vessel areas, NS	1988	Ernst et al. 1999

Once TBT has been introduced into the marine environment, it rapidly distributes among the surface microlayer, the water column and the surface layer of bottom sediments. Because of its low water solubility, TBT preferentially binds to suspended organic matter and bottom sediments (Laughlin et al. 1986). In a simulated marine microcosm, concentrations of TBT adsorbed on the suspended particles were three orders of magnitude greater than in the dissolved form. TBT was mainly removed from the water column by degradation to DBT and MBT and by partitioning to sediment, with 70% of total butyltins found in the sediment by the fourth day (Dai

et al. 1998). The strength of TBT sorption to sediments was demonstrated in a simulated dredging experiment, in which desorption of TBT from suspended sediments was very slow (Svavarsson et al. 2001). In our study, the liquid fraction of sediment samples from all Nova Scotia and St. John's Harbour locations contained very little TBT (concentrations near or below detection limits, detection limit in pore water of 0.5 ng/mL, sediment 1 ng/g, and mussel tissue 1 ng/g), even in samples with a high TBT concentration in the solid fraction.

While the half-life of TBT in water is usually just a matter of days or weeks, it can persist for years in marine sediment. Several studies that estimated TBT persistence from sediment core data revealed half-lives ranging from 1.85 to 15 years (Maguire 2000). TBT degradation products are also persistent; in a study that reported a half-life for TBT of 2.1 years, half-lives for DBT and MBT were 1.9 years and 1.1 years respectively (Sarradin et al. 1995).

Remnant TBT concentration in sediment is often used as a general measure of the extent of TBT degradation at a given location. When the TBT:total butyltin ratio is close to 100%, it indicates little degradation of TBT and/or recent introduction of TBT into the ecosystem (Chau et al. 1997a). A study of several Canadian harbours revealed a wide range of ratios, up to a maximum of 100%, and averaging 59% (Chau et al. 1997a). This average is similar to those found in several other large vessel harbours, ranging from 53% (Garrett & Shrimpton 1998) to over 70% (e.g. Page et al. 1996a). In this study, TBT percentages ranged from 33-88% (mean 50%) in Halifax Harbour sediments and 0-83% (mean 41%) in St. John's Harbour sediments (Table 4, Figure 9). In the 2003 Halifax Shipyard samples, the percentage was even higher, ranging from 94.2 to 97.4%. The higher percentages at Pier 18 in St. John's, and particularly at Halifax Shipyard in Halifax Harbour may indicate recent inputs of TBT.

The TBT ratio was generally lower at South Shore Marina (29%) and Oak Island Marina (31%), as would be expected in small vessel harbours where TBT inputs are older, due to restrictions placed on TBT-containing paints for smaller boats in 1989. These values are lower than percentages found by Ernst et al. at the same marinas in 1994 (43% at South Shore Marina, 32% at Oak Island Marina) and at a small vessel harbour in France (49%, Sarradin et al. 1994), suggesting that the 1989 regulations have at least reduced fresh inputs.

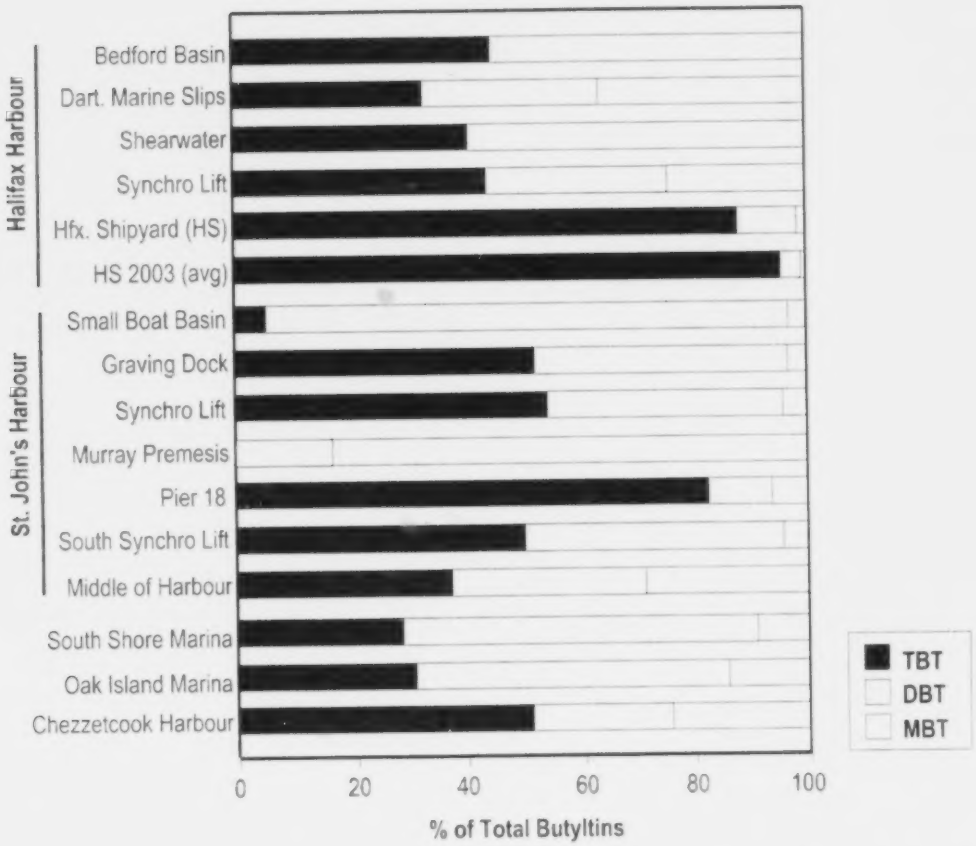

Figure 9. Tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) as a percentage of total butyltins in sediment.

3.2 Butyltins in Mussels

Concentrations of TBT in mussel samples were similar in the two harbours, ranging from 1.6-3.6 ng Sn/g wet weight in St. John's Harbour and 1.4-4.8 ng/g in Halifax Harbour (Table 7, Figure 10). No butyltin residues were detected in Chezzetcook Inlet mussel samples. The concentrations in St. John's and Halifax Harbour mussels were two to three orders of magnitude lower than the 143-1198 ng/g dw found in Halifax Harbour mussels by other researchers in 1995 (Chau et al. 1997b, Table 8). They were also lower than concentrations found on the west coast of Canada and in other countries in the past 15 years. This is in contrast to the sediment TBT concentrations in our study, some of which were among the higher values reported elsewhere (Table 8).

Table 7. Butyltin concentrations in mussels (*Mytilus edulis*) collected from St. John's, Halifax and Chezzetcook Harbours.

Location	Butyltin (ng Sn/g wet weight)			Total Butyltin	% TBT of Total
	TBT	DBT	MBT		
Halifax Harbour					
Bedford Yacht Club (BY)	2.5	16.7	3.2	22.4	11.2
Office (OF)	2.3	17.9	8.8	29.0	7.9
Dartmouth Cove (DC)	1.4	21.7	6.0	29.1	4.8
BIO (BI)	4.8	39.5	7.2	51.5	9.3
N. Container Pier (NC)	3.7	11.7	4.9	20.3	18.2
S. Container Pier (SC)	2.8	11.1	6.9	20.8	13.5
Halifax Harbour Mean	2.9	19.8	6.2	28.9	10.8
St. Dev	1.2	10.5	1.9	11.8	4.7
St. John's Harbour					
SJH-Pier 26 (26)	3.2	4.0	1.0	8.2	39.0
SJH-Hay Cove (HC)	1.6	7.5	1.9	11.0	14.5
SJH-Pier 3 (P3)	3.6	4.9	1.0	9.5	37.9
St. John's Harbour Mean	2.8	5.5	1.3	9.6	30.5
St. Dev	1.1	1.8	0.5	1.4	13.8
Chezzetcook Inlet (CZ) (control)	<1.0	<1.0	<1.0		

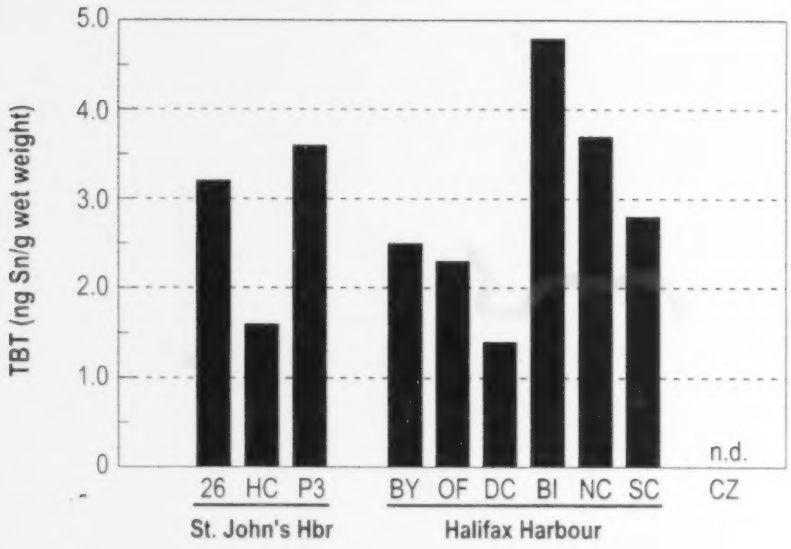

Figure 10. TBT concentrations in mussels.

Table 8. Mussel butyltin concentrations measured in this study and in others.

Concentration (ng Sn/g)*			Location (# sample locations or events)	Year(s)	Reference
TBT	DBT	MBT			
1.6-3.6 ww	4-7.5 ww	1-1.9 ww	St. John's Harbour, NL (3)	2002	This study
1.4-4.8 ww	11-40 ww	3.2-8.8 ww	Halifax Harbour, NS (6)	2002	This study
143-1198 dw	81-1062 dw	76-708 dw	Halifax Harbour, NS (6)	1995	Chau et al. 1997b
20-76 dw	<20 dw	<20 dw	Saint John Harbour, NB (4)	1995	Chau et al. 1997b
271-787 dw	168-565 dw	84-600 dw	2 coastal harbours, BC (5)	1995	Chau et al. 1997b
2-27 ww	0.5-2.8 ww		Southwestern Iceland (8)	1993-4	Skarphédinsdóttir et al. 1996
369 dw	192 dw		River, UK	1993	Page 1995
nd-135 ww			Coast, Perth, Australia (35)	1991	Burt & Ebell 1995
4-566 dw	5-378 dw	<14-203 dw	West coast USA (32)	1989-90	Uhler et al. 1993
16-492 dw	5-194 dw	<14-95 dw	East coast USA (46)	1989-90	Uhler et al. 1993
nd-964 dw	nd-173 dw		Harbours, Maine, USA	1989	Page et al. 1995
8-99 ww	20-276 ww	14-81 ww	Wharves, Tokyo Bay, Japan (26)	1989	Higashiyama et al. 1991
21-144 ww			UK estuaries, harbours, marinas (13)	1989	Waite et al. 1991
53-944 dw	3-174 dw	6-277 dw	The Netherlands (4)	1988	Ritsema et al. 1991
16-1687 dw	16-1297 dw		Coastal Maine, USA (6)	1987-89	Page et al. 1996b

* dw = dry weight
ww = wet weight

TBT concentrations as a percentage of total butyltin averaged 31% in St. John's Harbour and 11% in Halifax Harbour (Figure 11). These values were lower than in several other studies on *M. edulis*; for example, Chau et al. (1997b) reported an average of 63% TBT for 15 sites, and a range of 40-49% for Halifax Harbour.

The bioavailability of TBT and its degradation products in marine environments is affected by factors such as water temperature, salinity and particulate content (Beaumont & Budd 1984). Bioconcentration factors (biota concentration: water concentration) for TBT may range from 1,500 to >200,000 in various bivalve species (Maguire 2000).

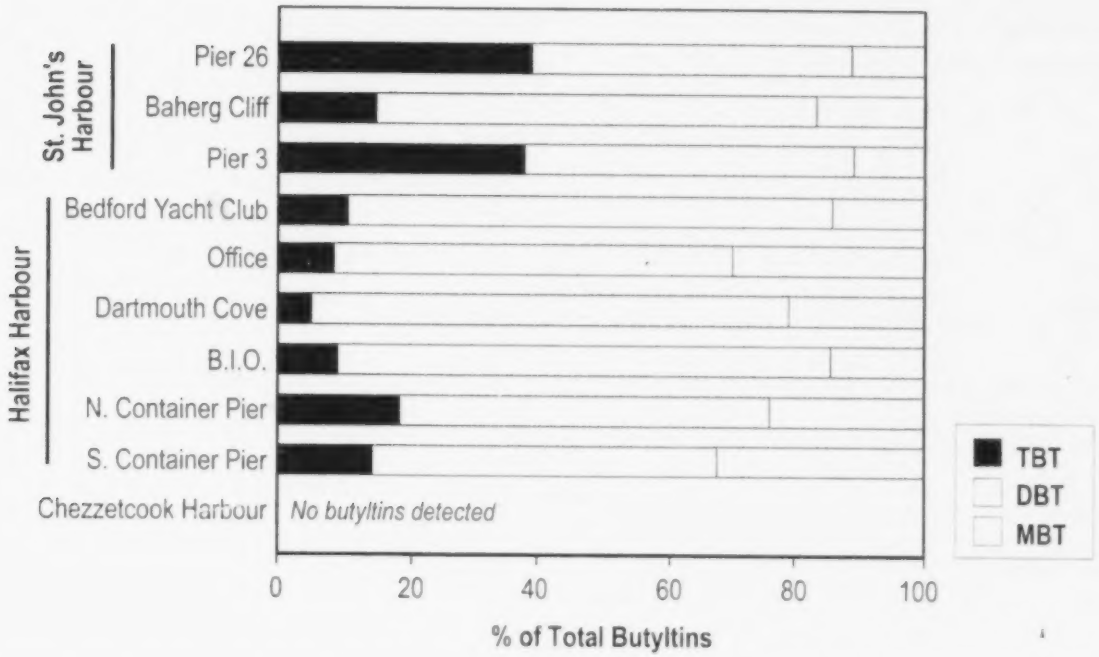

Figure 11. Tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) as a percentage of total butyltins in mussels.

Some deposit-feeding bivalves receive more TBT from phytoplankton than water (Laughlin et al. 1986b), while others accumulate more TBT from particulates than any other source (Langston & Burt 1991). For other organisms such as amphipods, however, sorption of TBT to sediments greatly reduces its bioavailability (Stronkhorst et al. 1999).

Toxic effects of TBT in mussels have only been documented at much higher concentrations than were found in this study. Page et al. 1991 reported no significant effects observable in adult mussels at 50-500 ng Sn/g dw, and significant effects such as changes in oxygen consumption, clearance rate and scope for growth at >2000 ng/g. These are similar to results reported by Salazar & Salazar (1991): possible effects on growth of juvenile mussels at 500-1500 ng/g, and probable effects at >1500 ng/g. More recent threshold levels have been 1640 ng Sn/g dry weight for probable effects on juvenile mussel growth (Salazar & Salazar 1998), and a site specific tissue trigger level for adult mussels of 600 ng/g wet weight or 3000 ng/g dry weight. At the low concentration of TBT found in our study, toxic effects have not been documented.

There is little data on the link between sediment TBT concentrations and mussel toxicity, although sediment concentrations lower than those found in this study have been shown to cause lethal and sublethal effects in other species (Langstrom & Burt 1991, Ruiz et al. 1994). Julien et al. 1991 reported a 15 day LC50 for *M. edulis* in a sediment concentration of 41,500 µg Sn/g, and 5,700 ng/g in a simulated sediment dumping exposure; concentrations that are much higher than in all but one sample found in our study. While several studies link water TBT concentrations to sublethal effects on mussels (Beaumont & Budd 1984, Jha et al. 2000), none addressing the sublethal effects of TBT concentrations in sediment were found.

concentrations to sublethal effects on mussels (Beaumont & Budd 1984, Jha et al. 2000), none addressing the sublethal effects of TBT concentrations in sediment were found.

Toxic effects of TBT in relation to sediment concentration have been studied in other organisms. Sediment TBT concentrations (dry weight) that have been linked to acute and chronic effects in European studies include: acute toxicity to the clam *Scrobiculara plana* at 10,000 ng Sn/g (Langston & Burt 1991); an LC50 for the amphipod *Corophium volutator* at 2,185 ng/g; an LC50 for the urchin *Echinocardium cordatum* at 1,594 ng/g (Stronkhorst et al. 1999); and chronic toxicity to *S. plana* at 300 ng/g (Ruiz et al. 1994). Given that 56% of sediment samples in our study exceeded 300 ng/g TBT, it is likely that there are toxic effects to benthic biota resulting from TBT concentrations in St. John's Harbour and Halifax Harbour.

3.3 Additional Metals in Sediments and Mussels

The heavy metal concentrations which were measured in sediment and mussels are listed in Tables 9-11. Correlations between metal concentrations, butyltin concentrations and biomarkers (mussels only) are tabulated in Appendix 1. The following discussion will focus on those metals/metalloids which were analyzed in both sediment and mussels and for which there are Canadian Environmental Quality Guidelines (CEQG) for Marine Sediment (CCME 2002): arsenic, cadmium, copper, lead and zinc.

3.3.1 Arsenic

Arsenic concentrations in St. John's Harbour sediment ranged from 5.6 to 28 µg/g dry weight. Eight out of nine samples exceeded the CEQG for marine sediment of 7.24 µg/g, indicating that biota may experience toxic effects at these sites. However, no samples reached the probable effects level of 41.6 µg/g.

Where the pleasure craft sites and the Halifax Harbour samples all exceeded the CEQG (by 2-3 times), none were as high as the probable effects level (PEL). However, one of the Halifax Shipyard samples collected in 2003 had a concentration of 217.9 µg/g, well above the PEL.

Table 9. Metal concentrations (µg/g dry weight) in sediments from St. John's Harbour.

Location	Metal Concentration (µg/g dry weight except % Fe)														
	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sb	Sn	V	Zn
CEQG TEL*		7.24	0.7		52.3	18.7					30.2				124
CEQG PEL**		41.6	4.2		160	108					112				271
Small Boat Basin W	2.1	10.4	<1.0	5.5	29.0	132.0	2.1	299.1	4.8	35.7	226.1	<1.0	9.8	138.1	163.7
Small Boat Basin E	0.5	5.6	<1.0	5.1	39.5	110.4	2.0	268.9	3.9	22.3	57.8	<1.0	5.1	89.8	77.4
Graving Dock	6.5	15.6	1.7	28.8	68.4	409.8	3.7	394.6	17.8	66.7	296.6	2.9	32.4	93.7	568.0
Synchro Lift	5.6	13.2	1.7	17.4	53.4	250.7	3.7	430.3	16.2	33.7	259.2	1.9	33.4	89.1	465.9
Murray Premises	19.4	27.7	2.9	10.2	62.6	268.9	2.5	358.6	21.6	27.6	361.1	2.1	41.5	98.0	469.0
Pier 18	6.7	14.5	2.2	9.0	46.9	182.4	2.8	309.3	24.8	24.2	427.8	3.3	50.0	106.5	351.2
S. Synchro Lift	3.5	14.9	1.3	25.6	74.9	314.2	4.3	441.4	12.2	48.6	364.7	4.6	28.0	88.1	553.4
Middle of SJH	8.9	17.2	2.7	13.5	56.1	228.3	2.7	365.6	20.1	28.5	363.0	2.9	42.9	96.4	439.7
Oceanex Pier	7.8	8.6	1.3	6.3	28.4	109.8	2.2	280.3	8.0	19.6	315.7	2.1	22.4	54.2	272.1

* Canadian Environmental Quality Guidelines threshold effects level (TEL) for marine sediment (µg/g dry weight).
** PEL = probable effects level.

Table 10. Metal concentrations (µg/g dry weight) in sediments collected from Nova Scotia sample sites in 2001/02 and 2003.

Location	Metal Concentration (µg/g dry weight except % Fe)														
	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sb	Sn	V	Zn
CEQG TEL*		7.24	0.7		52.3	18.7					30.2				124
CEQG PEL**		41.6	4.2		160	108					112				271
Bedford Basin	3.6	30.8	1.0	12.0	53.4	90.7	4.5	457.9	13.3	35.9	183.7	2.5		167.4	258.3
Dart. Marine Slips	2.0	34.4	<1.0	7.5	67.2	533.2	3.7	249.4	7.2	66.7	554.8	3.6		88.5	337.1
Shearwater	1.3	16.5	<1.0	7.8	37.2	64.4	3.6	415.5	5.8	31.9	67.3	0.9		74.5	179.8
Synchro Lift	6.8	24.7	1.2	10.7	61.8	189.1	3.7	439.1	11.5	38.2	197.6	3.3		103.0	384.8
Halifax Shipyard	3.2	32.9	1.3	38.9	74.2	526.1	5.0	518.2	14.6	100.3	361.1	14.6		90.1	1429.1
Hfx. Shpyd. 2003 #1		17.8	<1.0	9.2	41.3	217.3	2.8	717.7	7.9	25.5	270.3	3.7	20.0	54.2	518.5
Hfx. Shpyd. 2003 #2		21.5	<1.0	21.2	41.2	606.6	3.3	202.1	29.7	45.9	196.4	5.3	30.4	40.7	839.3
Hfx. Shpyd. 2003 #3		217.9	1.5	162.7	133.0	2592	16.1	922.5	100.3	277.0	2666	75.9	158.0	41.3	17769
Hfx. Shpyd. 2003 #4		20.1	<1.0	9.3	33.2	301.7	2.8	257.7	8.1	23.5	418.4	3.0	67.8	57.2	443
South Shore Marina	0.1	21.5	<1.0	2.7	27.4	251.9	0.9	200.1	4.2	14.5	41.2	0.4		22.5	86.1
Oak Island Marina	0.2	13.4	<1.0	3.1	21.2	120.9	2.1	298.4	3.8	48.5	48.8	0.3		36.8	90.1
Chezzetcook Hbr.	0.2	4.5	<1.0	4.9	21.5	8.1	1.7	398.4	0.9	12.2	8.6	0.2		32.9	35.6
Chez. Hbr. 2003 #1		<5.0	<1.0	2.9	11.1	5.0	<2.5	235.0	<1.0	6.7	4.8	<1.0	<1.0	20.1	21.8
Chez. Hbr. 2003 #2		<5.0	<1.0	2.9	10.4	4.7	<2.5	223.6	<1.0	6.7	4.8	<1.0	<1.0	21.1	19.8

* Canadian Environmental Quality Guidelines threshold effects level (TEL) for marine sediment (µg/g dry weight).
** PEL = probable effects level.

Table 11. Metal concentrations (µg/g dry weight) in mussel samples from St. John's, Halifax and Chezzetcook Harbours.

Location	As	Cd	Co	Cu	Mn	Ni	Pb	Se	Zn
St. John's Harbour									
Pier 26	5.76	0.51	0.51	29.20	20.26	13.56	8.27	3.19	144.1
Hay Cove	6.05	0.46	0.38	10.92	7.30	3.73	4.23	2.43	97.1
Pier 3	6.47	0.80	0.53	22.47	19.51	3.83	5.28	2.94	159.3
Halifax Harbour									
Bedford Yacht Club	6.94	0.48	0.59	6.08	19.22	0.87	0.80	1.91	71.9
Office	10.64	0.71	0.42	8.93	11.18	1.51	3.33	2.59	132.9
Dartmouth Cove	9.51	0.72	0.49	8.86	12.57	2.93	5.40	2.46	110.5
BIO	10.65	0.67	0.66	9.40	19.74	1.16	1.83	2.33	132.4
N. Container Pier	7.92	0.76	0.51	7.08	8.78	1.08	1.02	2.16	102.3
S. Container Pier	7.96	0.89	0.55	8.16	19.64	1.54	1.48	2.53	115.5
Chezzetcook Harbour	8.74	0.975	1.05	6.04	53.02	3.125	2.815	<0.25	46.6

Table 12. Comparison of sediment heavy metal concentrations (µg/g dry weight) in this study (not including 2003 samples) and other studies.

Location (n=)	As		Cd		Cr		Cu		Pb		Zn		Reference
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	
St. John's Harbour (9)	5.6	28	<1	2.9	28	68	110	410	58	427	77	568	This study
Halifax Harbour (5)	17	34	<1.0	1.3	37	74	61	533	67	555	179	1429	This study
NS Marinas (2)	13	21	<1.0	<1.0	21	27	121	252	41	49	86	90	This study
Chezzetcook Hbr (1)	4.5	4.5	<1.0	<1.0	22	22	8.1	8.1	8.6	8.6	36	36	This study
Halifax Harbour (250)			0.8 (mean)		12 (mean)		58 (mean)		160 (mean)		226 (mean)		Buckley & Winters 1992
Thames estuary, UK (84)		45		9.8		240		348		1634		1050	Attrill & Thomas 1995
UK estuaries (19)	5	1740	0.1	2.2	28	207	7	2398	20	2753	46	2821	Bryan 1992
Belgian North Sea (20)			0.3	0.7	26	105	1	5	5	30	12	70	Alsenoy et al. 1993

Table 13. Comparison of mussel tissue heavy metal concentrations (µg/g dry weight) in this study and other studies.

Location (n=)	As		Cd		Cu		Pb		Zn		Reference
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	
St. John's Harbour (9)	5.8	6.5	0.5	0.8	11	29	4.2	8.3	97	159	This study
Halifax Harbour (5)	6.9	11	0.5	0.9	7.1	9.4	0.8	5.4	72	133	This study
Chezzetcook Hbr (1)	8.7	8.7	1.0	1.0	6.0	6.0	2.8	2.8	47	47	This study
Halifax Harbour (3) (wet weight)	1.9	2.5	0.15	0.29	1.5	2.4	0.4	2.2	21	27	Hellou et al. (in press)
US Coasts (50)*	8.5	10	2.1	3.1	8.1	10	1.7	2.2	120	140	O'Connor et al. 1996
Gulf of Maine (56)**			1.57	2.47	6.8	8.7	0.7	2.6	96	120	Chase et al. 2001

* Ranges are average concentrations for 1986-93 in M. edulis (for Cu, Pb, Zn) and M. edulis plus other mollusks (for As, Cd).
** Ranges are median concentrations in M. edulis for 1991-1997.

This was also the sample with the highest butyltin concentrations. Chezzetcook Inlet had the only sample in Nova Scotia below the CEQG, at <5.0 µg/g.

Concentrations of arsenic in this study were similar to those found in a contaminated estuary in the UK (see Table 12). Thames estuary sediments averaged 15 µg arsenic/g, with a maximum of 45 µg/g (Attrill & Thomes 1995). An estuarine study throughout the UK demonstrated that arsenic concentrations could reach over 1700 µg/g in areas with significant pollution inputs (Bryan 1992). The main source of arsenic in water, however, is the weathering of rocks and ores which releases inorganic arsenic into surface water and runoff (Bryan 1992). Once in the marine environment, arsenic adsorbs strongly to sediments (mainly iron and manganese oxides), with its bioavailability inversely related to iron content. Sediment, as opposed to water, is the major source of arsenic to benthic invertebrates such as polychaetes and bivalves. In this study, concentrations of arsenic in mussels ranged from 5.8-6.5 µg/g dry weight in St. John's Harbour and 6.9-10.7 µg/g in Halifax Harbour. In Chezzetcook mussels, the arsenic concentration was similar (8.7 µg/g) indicating that the main sources of arsenic to the Atlantic Region marine environment are not anthropogenic. With all mussel samples grouped together, positive correlations ($p < 0.05$) were found between arsenic and total butyltins and DBT. Similar concentrations of arsenic were found in mollusks on the east and west coasts of the United States, where averages ranged from 8.5-10 µg/g (O'Connor et al. 1996).

3.3.2 Cadmium

Cadmium concentrations in St. John's Harbour sediment ranged from <1.0 to 2.9 µg/g dry weight. While the CEQG for cadmium of 0.7 µg/g is below the method detection limit for this study, at least seven out of nine samples exceeded the guideline. No samples reached the probable effects level of 4.2 µg/g.

Halifax Harbour sediment generally had lower concentrations of cadmium, ranging from <1.0 to 1.3 µg/g, with at least three samples exceeding the CEQG for marine sediment (but not the probable effects level). Cadmium was not detected in sediment from the remaining three sites. Only one of the 2003 samples exceeded the CEQG, but not the 1.5 µg/g PEL.

Concentrations of cadmium in this study were similar to those found in the late 1980s at 250 locations in Halifax Harbour, where the average concentration was 0.8 µg/g (Buckley & Winters 1992). They were also within the range of concentrations found in UK estuaries, although the maximum in the Thames estuary reached 9.8 µg/g (Table 10). In sediment, the most bioavailable form of cadmium is the divalent form, although it is not well absorbed when associated with iron oxides or bacteria. Biota accumulate cadmium from either interstitial water or sediment, with the main route being dissolved cadmium that has been remobilized from sediment (Bryan 1992). Cadmium may be genotoxic to mussels at water concentrations greater than 184 µg/L (Bolognesi et al. 1999). In this study, concentrations of cadmium in mussels ranged from 0.46 to 0.80 µg/g in St. John's Harbour and 0.48 to 0.89 µg/g in Halifax Harbour, with, inexplicably, the highest concentration found in Chezzetcook mussels (1.0 µg/g). Average cadmium concentrations were higher in mussels from US coasts and the Gulf of Maine (Table 11).

3.3.3 Copper

Copper concentrations in St. John's Harbour sediment ranged from 110 to 410 µg/g dry weight. All nine samples exceeded the CEQG (18.7 µg/g) and probable effects level (108 µg/g) indicating that biota are probably experiencing toxic effects at these sites.

Concentrations of copper in Halifax Harbour sediment had a wide range of 64 to 533 µg/g. One of the Halifax Shipyard samples collected in 2003 had an exceptionally high copper concentration of 2592 µg/g. This sample also had the highest concentration of TBT and other metals. Although somewhat lower, the South Shore and Oak Island Marina concentrations (252 and 121 µg/g) as well as the Halifax Harbour samples all exceeded the CEQG, with nine out of eleven above the probable effects level. Chezzetcook Inlet had the only samples in this study below the CEQG, ranging from 4.7 to 8.1 µg/g.

Concentrations of copper in this study were mostly higher than those found previously in Halifax Harbour and in the UK, and maximum concentrations were exceeded by only two sites in the UK (Table 12). A significant source of copper in the environment is the leaching of copper oxides from anti-fouling paints (Voulvoulis et al. 1999). The most toxic and bioavailable form of copper is the cupric ion, and the 48-hour EC_{50} for *M. edulis* in water is 5 µg Cu/L (Bryan 1992), with DNA damage noted at over 40 µg $CuCl_2$/L (Bolognesi et al. 1999). However, in the marine environment, copper is sensitive to complexation, and in sediment becomes associated with iron oxides and humic content. While the copper concentration in biota has been correlated to sediment concentration in many studies, this form of copper is less bioavailable and toxic. Increased tolerance of organisms to copper is also provided by binding with metallothioneins and Voulvoulis et al. (1999) stated that increased copper in the aquatic environment due to the use of copper-based antifoulants does not appear to have significant effects on marine ecosystems.

In this study, concentrations of copper in mussels ranged from 11-29 µg/g dry weight in St. John's Harbour and 6.1-9.4 µg/g in Halifax Harbour. In Chezzetcook mussels, the copper concentration was near the Halifax minimum, at 6.0 µg/g. While concentrations of copper in Halifax Harbour mussels were similar to those found in the US and the Gulf of Maine, the St. John's Harbour maximum was three times as high (Table 11).

3.3.4 Lead

Lead concentrations in St. John's Harbour sediment ranged from 58 to 427 µg/g dry weight. All nine samples exceeded the CEQG for marine sediment of 30.2 µg/g, and eight exceeded the PEL, indicating that biota are probably experiencing toxic effects due to lead at these sites.

Halifax Harbour sediment generally had similar concentrations of lead, ranging from 67-555 µg/g. Again, one Halifax Shipyard sample had a very high concentration of 2666 µg Pb/g. The South Shore and Oak Island Marina site concentrations were 41 and 48 µg/g respectively. These and the Halifax Harbour samples all exceeded the CEQG, and eight of the Halifax samples exceeded the probable effects level. The Chezzetcook Harbour samples were well below the CEQG, at 4.8 to 8.6 µg/g.

Lead concentrations in this study were higher than mean concentrations found previously in Halifax Harbour and the UK, although maximum concentrations in one UK study exceeded 2,000 µg/g (Table 10). Much of the lead in the marine environment may be attributed to the

past widespread use of leaded fuels (Bryan 1992) and lead pipes. The most toxic forms of lead are the free ion Pb^{2+} and organolead species. Many studies have shown that sediment lead is available to benthic organisms, and its uptake affected by factors such as the organic content of sediments, as well as the amount of free lead in interstitial water (Bryan 1992). In this study, concentrations of lead in mussels ranged from 4.2-8.3 µg/g dry weight in St. John's Harbour and 0.8-5.4 µg/g in Halifax Harbour. In Chezzetcook mussels, the lead concentration was in the same range, at 2.8 µg/g, which may be partially attributed to lead shot in Chezzetcook as a result of hunting activities. In general, concentrations of lead in mussels from Halifax and St. John's harbours were higher than on the US coasts and in the Gulf of Maine (Table 13).

3.3.5 Zinc

Zinc concentrations in St. John's Harbour sediment ranged from 77 to 568 µg/g dry weight. Eight out of nine samples exceeded the CEQG for marine sediment of 124 µg/g, and six were over the PEL, indicating that biota are likely experiencing toxic effects due to zinc at these sites.

Halifax Harbour sediment generally had a broader range of zinc concentrations, from 179 to 1429 µg/g in 2002, and up to 17,769 µg/g at Halifax Shipyard in 2003. All nine samples exceeded the CEQG of 124 µg/g and seven were over the PEL of 271 µg/g. The South Shore Marina, Oak Island Marina and Chezzetcook sediment concentrations were all below the CEQG.

Zinc concentrations in this study were comparable to those found previously in Halifax Harbour and the UK, although maximum concentrations in one UK study exceeded 2500 µg/g (Table 10). The most abundant and bioavailable form of zinc is the free ion Zn^{2+}, and as with arsenic and lead, the metal ion becomes less available as it binds to iron oxides in sediment. Unlike other metals, tissue zinc concentrations tend to be independent of concentrations in water and sediment. While toxic exposures may affect fertility of benthic organisms, very high tissue concentrations have been observed with little obvious effect. This may be due to decreased permeability to zinc in tolerant organisms, as well as increased metallothionein binding. In this study, concentrations of zinc in mussels ranged from 97-159 µg/g dry weight in St. John's Harbour and 72-133 µg/g in Halifax Harbour. In Chezzetcook mussels, the zinc concentration was significantly lower, at 47 µg/g. In general, concentrations of zinc in mussels were similar on the US coasts and in the Gulf of Maine (Table 11).

3.4 Biomarker Studies

3.4.1 Condition Index

Table 14 summarizes the results of biomarker analyses. The mussels collected from Halifax Harbour generally had a greater condition index length and weight (2.2-2.6 g/cm), than those from St. John's Harbour (1.1-1.7 g/cm). Grouping all sites together, condition index had a significant ($p = <0.05$) negative correlation with tissue copper concentrations ($R = -0.84$) and a positive correlation with tissue DBT ($R = 0.83$) and arsenic ($R = 0.82$). All other concentrations of metals were not significantly correlated with condition index.

3.4.2 Vitellin-like Proteins

Concentrations of vitellin-like proteins (Vn) were similar at both sites, ranging from 0.19-0.30 µg/mg in Halifax mussels and 0.20- 0.28 µg/mg in St. John's mussels (Table 14). A concurrent

study of mussels collected between October and January from Saint John Harbour, NB showed Vn levels with greater variation (0.04-0.67 µg/mg), which may be due in part to differing levels of reproductive activity between October and January (Gagné 2003). The results from December and January (0.16 and 0.04 µg/mg respectively) were lower than those from Halifax and St. John's collected around the same time. No significant correlations were found between Halifax and St. John's Harbour mussel Vn and metal/butyltin concentrations.

Concentrations of Vn in bivalves has only recently begun to be measured as a marker of endocrine disrupters in the environment. Vitellin-like proteins are similar to vitellogenin (Vg), proteins that are produced by oviparous organisms in response to estrogen stimulation of Vg genes in the liver. In fish, Vg proteins supply important energy reserves for fertilized eggs and yearlings. Although male fish do not produce Vg, they have a functional Vg gene that can be activated by estrogen and xenoestrogens (Flouriot et al. 1995, cited in Blaise et al. 1999). Blaise et al. 1999 developed a method for the indirect measurement of vitellin-like proteins in the soft-shell clam, *Mya arenaria* through an alkali-labile phosphate (ALP) assay. This method has been adapted for use in other bivalves, including *Mytilus edulis*.

Table 14. Results of biomarker analyses* of the digestive glands and/or gonads from mussels collected at Halifax and St. John's Harbours.

Mussel Sample Location	Weight g	Length cm	CI g/cm	Vn µg/mg	MT µg/mg	Zn gon ng/mg	Zn dg ng/mg	LPO µg/mg
HH – BYC	10.20	4.40	2.32	0.295	0.032	3.50	0.96	4.10
	±1.80		±0.27	±0.080	±0.011	±1.51	±0.61	±1.08
HH – Office	12.05	4.73	2.55	0.193	0.026	6.41	1.02	2.45
	±1.79		±0.33	±0.057	±0.011	±2.80	±0.90	±0.76
HH – Dart Cove	9.75	4.53	2.15	0.290	0.038	5.59	1.98	4.23
	±1.25		±0.38	±0.158	±0.020	±3.80	±0.91	±0.92
HH – BIO	11.73	4.49	2.61	0.280	0.029	3.03	1.82	2.71
	±2.20		±0.33	±0.082	±0.014	±0.82	±1.05	±0.81
SJH – Pier 26	5.85	4.50	1.30	0.196	0.031	12.25	8.54	3.37
	±1.50		±0.23	±0.050	±0.020	±6.17	±2.50	±1.41
SJH – Baherg	4.65	2.70	1.72	0.283	0.042	14.98	8.97	2.00
	±0.90		±0.32	±0.092	±0.023	±6.15	±3.20	±0.52
SJH – Pier 3	3.60	3.36	1.07	0.262	0.027	7.22	4.10	4.03
	±0.85		±0.22	±0.105	±0.010	±3.30	±1.54	±1.12

* **CI** = condition index; **Vn** = relative concentrations of vitellin-like proteins in gonads (µg alkali-labile phosphate/mg gonad proteins); **MT** = concentrations of metallothioneins in digestive glands (µg thiols/mg digestive gland proteins); **Zn gon/Zn dg** = labile zinc concentrations in gonads and digestive glands (ng Zn/mg proteins); **LPO** = lipid peroxidation in digestive glands (µg thiobarbituric acid reactans [malonaldehyde]/mg proteins).

During the past few years, Vn elevations in bivalves from contaminated sites have been linked to the presence of estrogenic compounds from industrial effluents, sewage effluents, and pesticide migration (Blaise et al. 1999, Gagné et al. 2001a, Gaulthier-Clerc et al. 2002). Alternately, decreased concentrations of Vn may be due to reduced nutrient levels, exposure to anti-estrogenic chemicals and metals such as cadmium, and the time of year (levels tend to be highest in May and June) (Blaise et al. 1999, Olsson et al. 1995). Without regional background concentrations, as well as a shortage of data in the literature pertaining to Vn concentrations in mussels during the winter months, it is difficult to put the results for the Halifax and St. John's Harbour mussels into perspective. Further studies, including collection and analysis of mussels from non-polluted areas during December and January, would aid in determining whether our results are above or below expected values.

3.4.3 Metallothioneins

Metallothionein (MT) concentrations were similar at the Halifax (0.026-0.038 µg/mg) and St. John's (0.027-0.042 µg/mg) sites. When data was grouped with results from Saint John's Harbour, a significant negative correlation was found between MT and Vn ($R = 0.40$, $p<0.01$). No correlations were found between Halifax and St. John's MT concentrations and metal concentrations in mussel tissues.

MT and MT-like proteins are measured as markers of environmental stress as they play a key role in trace metal metabolism and detoxification mechanisms (Pavicic et al. 1993). Increased tissue concentrations of MT are found when organisms are exposed to bioavailable heavy metals such as copper, zinc, cadmium, lead and silver (Pavicic et al. 1993, Viarengo et al. 1997). Additionally, condition index or weight may influence MT levels in bivalves (Cosson 2001). Exposure to xenoestrogens can also affect MT levels through interactions with vitellogenesis. For example, increased vitellogenesis in fish suppresses MT production as the zinc and copper normally bound by MT are needed for vitellin synthesis. While this relationship has yet to be confirmed in mussels (Gagné, pers. comm.), a significant negative correlation was found between the two parameters in this study. Nonetheless, analysis of covariance for this study indicated that site factors had an effect on MT levels that is not related to either mussel weight or Vn.

3.4.4 Labile Zinc

Labile zinc concentrations were higher in St. John's Harbour mussels (gonads 7.2-15.0 ng/mg, digestive glands 4.1-9.0 ng/mg) than in Halifax Harbour mussels (gonads 3.0-6.4, digestive glands 1.0-2.0 ng/mg). This assay measures the available form of the essential metal zinc in tissues and has been proposed as a biomarker of exposure to heavy metals. Recent studies have found elevated labile zinc concentrations in biota from contaminated sites (e.g. Gagne et al. 2002). As zinc is bound by MT and is required for vitellogenesis, its levels may be modulated by these factors. When results from this study were grouped, an analysis of covariance showed that labile zinc levels are more influenced by Vn in the digestive gland than in the gonad.

3.4.5 Lipid Peroxidation

Similar levels of lipid peroxidation (LPO) were found in the digestive glands of mussels from Halifax Harbour (2.5-4.2 µg/mg) and St. John's Harbour (2.0-4.0 µg/mg). No significant correlations were found between LPO and tissue concentrations of metals or butyltins. The

LPO biomarker measures oxidative damage, particularly within the polyunsaturated lipid-rich cell membranes (Wills, 1987). This form of damage may be triggered by various compounds, and is not specific to particular chemical classes. Increased LPO levels may be induced by heavy metal exposure, while MT production may reduce oxidative damage. When grouped with Saint John's Newfoundland samples, LPO results were negatively correlated ($p<0.05$) with MT concentrations as well as mussel condition indices.

4.0 SUMMARY AND CONCLUSIONS

This study assessed the status of TBT contamination in Atlantic Canada harbours in the year before sales of TBT antifoulants ceased. TBT concentrations in Halifax Harbour sediments were highly variable (42 to over 46,000 ng Sn/g) and were higher than those previously measured at the same location in approximately 50% of the sites. Most sample concentrations were similar to those found elsewhere in Canada and other countries, with the exception of the highest value, which was found at Halifax Shipyard. Sampling at Halifax Shipyard a year later yielded a mean TBT concentration (5,639 ng Sn/g) which, although less than the 2002 result, was still among the higher concentrations found worldwide. St. John's Harbour TBT results were generally lower than those in Halifax (<1.0 to 866 ng Sn/g). At some locations in both harbours, sediment TBT concentrations as a percentage of total butyltins indicate recent inputs of TBT into the marine environment. The mussel TBT concentrations in both harbours (1.6-4.8 ng Sn/g) were much lower than in other years, which may be reflective of condition status of the animals, however the elevated sediment concentrations indicate that the risk to benthic organisms remains high. Now that a ban has been placed on the selling and use of TBT-based paints on larger vessels, TBT concentrations in the marine environment are expected to gradually decline.

Concentrations of TBT were generally lower at the pleasure craft marinas (43-212 ng/g) than in the large vessel harbours, and similar to those found at marinas elsewhere. However, concentrations were not lower than in samples collected at the same locations in 1988 and 1994. Although the use of TBT-based paints on smaller boats has been restricted for several years, this may reflect an increase in boat traffic in the area, or the continued use of stock piled coatings. It is expected that these concentrations will eventually decline as stocks of TBT loadings are depleted.

Analyses of additional parameters in sediment and mussels were conducted to measure other indicators of pollution and determine if any correlations existed with butyltin levels. Concentrations of several metals in sediments (many exceeding Canadian Environmental Quality Guidelines and probable effects levels) were similar to those found in previous years and other locations, were not well correlated with TBT concentrations. Various mussel biomarkers that may indicate effects of contaminant exposure were measured, however, in the absence of baseline mussel data for that time of year, it was not clear if measurements were above expected levels. Further testing, particularly of mussels from non-polluted areas, is recommended to put these results into perspective.

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APPENDIX